MICROORGANISMS ASSOCIATED WITH DENTAL CARIES AMONG CHILDREN AND TEENAGERS

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ABSTRACT

Tooth decay, also known as dental caries is an epidemic, microbiological contagious disease of the teeth that ends in localized dissolution and damage of the calcified structure of the teeth. This disease occurs due to multiple factors such as interactions within the plaque community, host physiology, diet, fluoride and the nature of the tooth enamel, and dominance of *Mutans streptrococci*. In spite of development in science of oral diseases, dental caries extend to be a global health concern affecting human being of different age groups especially children and teenagers. With this concern, this research highlights different microbiological perspectives of dental caries affecting children and teenagers in a broader sense, it also presents the causes, signs and symptoms, diagnosis, treatment and prevention of dental caries in children and teenagers, and recommendations from this research will help to upgrade the recent trends of microbiology in dental caries and also formulating various developmental programs towards oral hygiene.

Keywords: Dental Caries, disease, global health.

1.0 INTRODUCTION

The word caries is derived from the Latin word meaning rot or rotten. According to Bader, dental caries is a chronic contagious disease caused by a complete interaction of oral microorganism in dental plaque, diet and a broad array of host factors ranging from societal & environmental factors to genetic & biochemical/immunologic host responses (Petersen *et al.*, 2005).

Dental caries is one of the most prevalent chronic diseases in man, worldwide. It is a multifactorial disease that starts with microbiological shifts within the complex biofilm (dental plaque). Caries is affected by the consumption of dietary sugars, salivary flow, exposure to fluoride and preventive behaviours (Selwitz *et al.*, 2007). Epidemiological studies have reported a decline in caries among children and teenagers in Western Europe during the last decades (Marthaler, 2004; Downer *et*

al., 2005; Hugoson *et al.*, 2008). However, among pre-school children, a tendency towards a stagnation in caries decline has been reported since the end of the 1980s (Hugoson *et al.*, 2005; Stecksén-Blicks *et al.*, 2004).

Furthermore, there is a trend in many developed countries for the prevalence of dental caries to increase again, especially among young children (Haugejorden & Birkeland, 2002). As a result, dental caries is still common among children and adolescents (Nithila *et al.*, 1998; Marthaler, 2004) and affects 46% of 4- year-old children (Stecksén-Blicks *et al.*, 2004) and 80% of 15-year-olds (Hugoson *et al.*, 2008). In the Jönköping study, the mean number of decayed and filled tooth surfaces (DFS), including initial lesions at 15 years of age, was 6.4, while the distribution by type of surface for the proximal, occlusal and buccal/lingual was 3.0, 1.5 and 1.9, respectively. Moreover, dental caries is a public health problem because it is a widespread condition that is costly to treat and has an impact on the quality of life of children of all ages (Low *et al.*, 1999; Filstrup *et al.*, 2003; Ismail, 2004). It is therefore of the utmost important to prevent the caries disease, but this will not be successful unless the available scientific knowledge relating to ways of changing the etiological factors of the disease is applied.

Dental caries is one of the most common chronic infectious diseases in the world (Anusavice, 2002). There are three major hypotheses for the etiology of dental caries: the specific plaque hypothesis, the nonspecific plaque hypothesis, and the ecological plaque hypothesis (Loesche, 1992). The specific plaque hypothesis has proposed that only a few specific species, such as *Streptococcus mutans* and *Streptococcus sobrinus*, are actively involved in the disease. On the other hand, the nonspecific plaque hypothesis maintains that caries is the outcome of the overall activity of the total plaque microflora, which is comprised of many bacterial species (Theilade, 1986). The ecological plaque hypothesis suggests that caries is a result of a shift in the balance of the resident microflora driven by changes in local environmental conditions (Marsh, 1994).

1.2 AIM AND OBJECTIVE

The aim of this project work is to study and provide a foundation knowledge on microorganisms associated with dental caries and to isolate microorganism associated with dental caries prevalent in human being.

The objectives of this work is to determine some bacterial species that are associated

with health and dental caries of permanent teeth in young adults.

1.3 STATEMENT OF PROBLEM

The experience of pain, problem with eating, chewing, smiling and communication due to missing, discoloured or spoiled teeth have a foremost impact on people's everyday life. Moreover, oral diseases hamper activities at school, at occupation and at residence causing millions of school and employment hours to be vanished each year throughout the globe. As a consequence, the treatment need in needed.

1.4 SCOPE OF STUDY

During the last few decades, the incidence of microbial disease has amplified drastically. Microorganisms are the super bug agent responsible for causing dental caries. Many facultatively and obligately anaerobic dominate the microbial community of dental caries. This work is limited to the identification of these microorganism among people with dental caries

2.0 LITERATURE REVIEW

2.1 HISTORICAL EVIDENCE OF CARIES IN EARLY HUMANS

There has been long history of dental caries. Caries is a very old disease and it is not exclusive of the human species. Evidences of dental lesions compatible with caries have been observed in creatures as old as: Paleozoic fishes (570-250 million years), Mesozoic herbivores dinosaurs (245-265 million years), prehominines of the Eocene (60- 25 million years), and Miocenic (25-5 million years), Pliocenic (5- 1.6 million years), and Pleistocenic animals (1.6-0.01 million years) Caries has also been detected in bears and other wild animals and it is common in domestic animals (Kemp, 2003).

The rate of caries remained low through the Bronze and Iron ages. The increase of caries during the Neolithic period may be attributed to the increase of plant foods containing carbohydrates. The beginning of rice cultivation in South Asia also believed to have caused an increase in caries. The earliest theory was the "tooth worm theory" proposed by the ancient Chinese in 2500 BC, where it posited a tooth worm as the cause of this rottenness. In 350 BC Aristotle observed figs and sweets caused tooth decay and by the 12th century, caries was described as the condition of having holes in the teeth or cavities. A Sumerian text from 5000 BC describes a "tooth worm" as the cause of caries (Forrai, 2009).

There is a long history of dental caries. Over a million years ago, hominins such as Australopithecus suffered from cavities.

The largest increases in the prevalence of caries have been associated with dietary changes (Suddick, and Harris, 1990) Archaeological evidence shows that tooth decay is an ancient disease dating far into prehistory. Skulls dating from a million years ago through the neolithic period show signs of caries, including those from the Paleolithic and Mesolithic ages. The increase of caries during the neolithic period may be attributed to the increased consumption of plant foods containing carbohydrates.The beginning of rice cultivation in South Asia is also believed to have caused an increase in caries especially for women, although there is also some evidence from sites in Thailand, such as KhokPhanom Di, that shows a decrease in overall percentage of dental caries with the increase in dependence on rice agriculture (Suddick, and Harris, 1990).

2.2 DEVELOPMENT OF DENTAL CARIES

Dental caries or cavities also called Tooth Decay, is a breakdown of teeth due to acids made by bacteria (Silk, 2014). The cavities may be a number of different colors from yellow to black. Symptoms may include pain and difficulty with eating. Complications may include inflammation of the tissue around the tooth, tooth loss, and infection or abscess formation (Laudenbach, and Simon, 2014)

The cause of caries is acid from bacteria dissolving the hard tissues of the teeth (enamel, dentin and cementum). The acid is produced by the bacteria when they break down food debris or sugar on the tooth surface. Simple sugars in food are these bacteria's primary energy source and thus a diet high in simple sugar is a risk factor (Section on Oral Health, 2014). If mineral breakdown is greater than build up from sources such as saliva, caries results. Risk factors include conditions that result in less saliva such as: diabetes mellitus, Sjogren's syndrome and some medications. Medications that decrease saliva production include antihistamines and antidepressants. Caries is also associated with poverty, poor cleaning of the mouth, and receding gums resulting in exposure of the roots of the teeth (Section on Oral Health, 2014).

Dental caries development is considered to involve a triad of indispensable factors: bacteria (dental plaque), carbohydrates (the diet), and susceptible teeth (the host) (Keyes andJordan, 1963). The essential process involves demineralization of tooth enamel, and likely also of root surfaces, by high concentrations of organic acids produced by bacteria in dental plaque from dietary carbohydrates. The microbiota of plaque is known to consist of a variety of acidogenic, non-acidogenic, and base producing organisms and to differ in

composition in different dentition sites. A crucial issue is whether caries induction does or does not reflect the activity of only certain specific acidogenic plaque bacteria (concept of specific vs. non-specific bacterial etiology). The former concept has been viable since the early 1900's. The first organisms to be implicated as specific cariogenic agents were the lactobacilli. Their demise, as major etiologic agents, was followed by a period dominated by the concept of non-specific bacterial etiology (MacDonald, 1960), whereas during the 1960's the concept of a specific bacterial etiology was revived with the re-discovery of Streptococcus mutans. At present, a few specific organisms in plaque are considered by many to play a special role in coronal caries development (for review, see Hamada and Slade, 1980; Loesche, 1986; Tanzer, 1989; van Houte, 1980). Less-detailed information suggests that a broader spectrum of bacteria is involved in root-caries development (Bowden, 1990;Jordan, 1986).

2.3 APPROXIMAL CARIES PREVALENCE IN PERMANENT TEETH

In Sweden, data relating to dental caries in individuals 3 to 19 years of age are available in annual reports from 1985 to 2005 and are given as the percentage of children who are free from manifest caries (Socialstyrelsen, 2006). However, initial caries lesions are not included in these data. Consequently, the national reports often underestimate the true prevalence of caries (Amarante *et al.*, 1998; Machiulskiene *et al.*, 1998). In studies by MobergSköld *et al.* (1995, 2005a, b), approximal initial lesions have been reported to constitute 80-90% of the total number of caries lesions in teenagers. This is in accordance with a study conducted by Forsling *et al.*, (1999). They studied the prevalence and distribution of approximal initial and manifest caries among 19-year-old Swedish patients and found that initial caries lesions constituted 90% of all lesions. Additionally, Poorterman *et al.*, (2002) reported from a Dutch population that 23% of the 14-year-olds were caries free on the approximal surfaces if initial (enamel) lesions were included. The corresponding value for 17-year-olds was 18%.

Owing to the potential risk of initial lesions progressing to manifest caries lesions (Mejàre *et al.*, 1999; David *et al.*, 2006), the prevalence of initial caries and its significance for further caries development ought to be a reason for finding new strategies for caries prevention. This is in line with Nyvad (2004), who concluded that "the time is now ripe for a move from operative to non-operative care in the management of dental caries with the

essential aims of arresting and healing the caries lesions at an early stage". The intention should be primary prevention, i.e. to prevent even non-cavitated approximal initial caries lesions (Raadal *et al.*, 2001; Pitts, 2004)

Furthermore, reporting approximal initial caries lesions is very important, since it shows the actual caries prevalence in the population. For the early diagnosis of approximal caries lesions, the bitewing radiograph is an important diagnostic tool (Kidd & Pitts, 1990; Espelid *et al.*, 2003; Clark & Curzon, 2004). According to Kidd and Pitts (1990), the bitewing radiograph is particularly important in the detection of the initial lesion, which may be managed preventively rather than operatively. There is, however, a need to ensure that radiographic exposures are minimised. The need for radiographs should therefore be balanced with the ethical issues associated with failing to make use of an established diagnostic aid. Lith and Gröndahl (1992) suggested that it ought to be possible to use previous caries experience as a method for individualising the planning of future radiographic procedures. More studies and regular national reports with special emphasis on approximal caries including initial caries lesions are needed to obtain a real picture of the caries situation in the permanent teeth.

2.4 CAUSES OF DENTAL CARIES

Four things are required for caries formation: a tooth surface (enamel or dentin), caries-causing bacteria, fermentable carbohydrates (such as sucrose), and time. This involves adherence of food to the teeth and acid creation by the bacteria that makes up the dental plaque. However, these four criteria are not always enough to cause the disease and a sheltered environment promoting development of a cariogenic biofilm is required. The caries disease process does not have an inevitable outcome, and different individuals will be susceptible to different degrees depending on the shape of their teeth, oral hygiene habits, and the buffering capacity of their saliva. Dental caries can occur on any surface of a tooth that is exposed to the oral cavity, but not the structures that are retained within the bone (Smith *et al.*, 1990)

Dental Caries is caused by biofilm (dental plaque) lying on the teeth and maturing to become cariogenic (causing decay). Certain bacteria in the biofilm produce acid in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose (Hardie, 1982)

Caries occur more often in people from the lower end of the socioeconomic scale than

people from the upper end of the socioeconomic scale.

2.4.1 BACTERIA

The most common bacteria associated with dental cavities are the mutans streptococci, most prominently *Streptococcus mutans* and *Streptococcus sobrinus*, and lactobacilli. However, cariogenic bacteria (the ones that can cause the disease) are present in dental plaque, but they are usually in too low concentrations to cause problems unless there is a shift in the balance. This is driven by local environmental change, such as frequent sugar, no biofilm removal (a lack of tooth brushing). If left untreated, the disease can lead to pain, tooth loss and infection (Marsh, 1994)

The mouth contains a wide variety of oral bacteria, but only a few specific species of bacteria are believed to cause dental caries: *Streptococcus mutans* and *Lactobacillus* species among them. *Streptococcus mutans* are gram-positive bacteria which constitute biofilms on the surface of teeth. These organisms can produce high levels of lactic acid following fermentation of dietary sugars and are resistant to the adverse effects of low pH, properties essential for cariogenic bacteria (Hardie, 1982). As the cementum of root surfaces is more easily demineralized than enamel surfaces, a wider variety of bacteria can cause root caries, including *Lactobacillus acidophilus*, *Actinomyces spp.*, *Nocardia spp.*, and *Streptococcus mutans*. Bacteria collect around the teeth and gums in a sticky, creamy-coloured mass called plaque, which serves as a biofilm. Some sites collect plaque more commonly than others, for example, sites with a low rate of salivary flow (molar fissures). Grooves on the occlusal surfaces of molar and premolar teeth provide microscopic retention sites for plaque bacteria, as do the interproximal sites. Plaque may also collect above or below the gingiva, where it is referred to as supra- or sub-gingival plaque, respectively.

These bacterial strains, most notably *S. mutans*, can be inherited by a child from a caretaker's kiss or through feeding premasticated.

2.4.2 SUGAR CONSUMPTION AS A CARIES-PROMOTING FACTOR

Dental caries has been defined as a dietary "carbohydrate–modified bacterial infectious disease" (van Houte, 1994). A sucrose-rich diet increases the growth rate of many oral bacteria and changes the composition of the microflora in a caries-promoting manner (Marsh & Nyvad, 2001). Acid-producing micro-organisms, such as

the mutans streptococci in dental plaque, play an essential role in the caries process (van Houte 1994; Caufield *et al.* 2005). The high and frequent consumption of sugar has been known to be an aetiological factor in caries for several decades (Moynihan *et al.*, 2003). However, a systematic review has shown that today, with frequent fluoride exposure, the relationship between sugar consumption and caries experience is not consistent (Burt & Pai, 2001). Sundin (1994) concluded that nowadays the consumption of sweets and other sugary products does not seem to be a strong factor for the occurrence of caries. However, for subjects with a combination of poor oral hygiene, the consumption of sweets has been shown to be particularly harmful. This is in line with Zero (2004), who pointed out that, in subgroups without the same fluoride protection, sugar still acts as a potential risk. Studies have also shown that the frequent consumption of caries-risk products, candy and sugar-containing beverages, particularly sweetened liquid in a feeding bottle, during the first years of life was associated with caries development during pre-school years (Wendt & Birkhed, 1995b; Grindefjord *et al.*, 1996). In addition, Guthrie and Morton (2000) showed that sweetened drinks constitute the primary source of added sugar in children's daily diet. Marshall *et al.*,(2003, 2007) have suggested that contemporary changes in beverage intake, particularly the increase in soda pop consumption, have the potential to increase dental caries rates in children. According to Sheiham (2001), "sugars, particularly sucrose, are the most important dietary aetiological cause of caries and the main strategy to further reduce the levels of caries, is reducing the frequency of sugars intakes in the diet". Continued action to promote good dietary habits is necessary for both dental and general health. However, more knowledge is needed about the establishment of dietary habits from early age and the effect on caries development later in life.

2.5 SIGNS AND SYMPTOMS OF DENTAL CARIES

A person experiencing caries may not be aware of the disease. The earliest sign of a new carious lesion is the appearance of a chalky white spot on the surface of the tooth, indicating an area of demineralization of enamel. This is referred to as a white spot lesion, an incipient carious lesion or a "microcavity". As the lesion continues to demineralize, it can turn brown but will eventually turn into a cavitation ("cavity"). Before the cavity forms, the process is reversible, but once a cavity forms, the lost tooth structure cannot be regenerated. A lesion that appears dark brown and shiny suggests dental caries were once present but the demineralization

process has stopped, leaving a stain. Active decay is lighter in color and dull in appearance (Clarke, 2007).

As the enamel and dentin are destroyed, the cavity becomes more noticeable. The affected areas of the tooth change color and become soft to the touch. Once the decay passes through enamel, the dentinal tubules, which have passages to the nerve of the tooth, become exposed, resulting in pain that can be transient, temporarily worsening with exposure to heat, cold, or sweet foods and drinks. A tooth weakened by extensive internal decay can sometimes suddenly fracture under normal chewing forces. When the decay has progressed enough to allow the bacteria to overwhelm the pulp tissue in the center of the tooth, a toothache can result and the pain will become more constant. Death of the pulp tissue and infection are common consequences. The tooth will no longer be sensitive to hot or cold, but can be very tender to pressure (Clarke, 2007).

Dental caries can also cause bad breath and foul tastes. In highly progressed cases, an infection can spread from the tooth to the surrounding soft tissues. Complications such as cavernous sinus thrombosis and Ludwig angina can be life-threatening (Clarke, 2007).

2.6 DENTAL CARIES DIAGNOSIS

The presentation of caries is highly variable. However, the risk factors and stages of development are similar. Initially, it may appear as a small chalky area (smooth surface caries), which may eventually develop into a large cavitation. Sometimes caries may be directly visible. However other methods of detection such as X-rays are used for less visible areas of teeth and to judge the extent of destruction. Lasers for detecting caries allow detection without ionizing radiation and are now used for detection of interproximal decay (between the teeth). Disclosing solutions are also used during tooth restoration to minimize the chance of recurrence (Rosenstiel, 2006).

Primary diagnosis involves inspection of all visible tooth surfaces using a good light source, dental mirror and explorer. Dental radiographs (X-rays) may show dental caries before it is otherwise visible, in particular caries between the teeth. Large areas of dental caries are often apparent to the naked eye, but smaller lesions can be difficult to identify. Visual and tactile inspection along with radiographs are employed frequently among dentists, in particular to diagnose pit and fissure caries. Early, uncavitated caries is often diagnosed by blowing air across the suspect surface, which removes moisture

and changes the optical properties of the unmineralized enamel (Rosenstiel, 2006).

Some dental researchers have cautioned against the use of dental explorers to find caries, in particular sharp ended explorers. In cases where a small area of tooth has begun demineralizing but has not yet cavitated, the pressure from the dental explorer could cause a cavity. Since the carious process is reversible before a cavity is present, it may be possible to arrest caries with fluoride and remineralizethe tooth surface. When a cavity is present, a restoration will be needed to replace the lost tooth structure (Zadik, and Ron, 2008).

At times, pit and fissure caries may be difficult to detect. Bacteria can penetrate the enamel to reach dentin, but then the outer surface may remineralize, especially if fluoride is present. These caries, sometimes referred to as "hidden caries", will still be visible on X-ray radiographs, but visual examination of the tooth would show the enamel intact or minimally perforated. The differential diagnosis for dental caries includes dental fluorosis and developmental defects of the tooth including hypomineralization of the tooth and hypoplasia of the tooth (Baelum *et al.*, 2008).

The early carious lesion is characterized by demineralization of the tooth surface, altering the tooth's optical properties. Technology utilizing laser speckle image (LSI) techniques may provide a diagnostic aid to detect early carious lesions (Deana, 2006).

2.7 TREATMENT

Most importantly, whether the carious lesion is cavitated or non-cavitated dictates the management. Clinical assessment of whether the lesion is active or arrested is also important. Non-cavitated lesions can be arrested and remineralization can occur under the right conditions. However, this may require extensive changes to the diet (reduction in frequency of refined sugars), improved oral hygiene (tooth brushing twice per day with fluoride toothpaste and daily flossing), and regular application of topical fluoride. Such management of a carious lesion is termed "non-operative" since no drilling is carried out on the tooth. Non-operative treatment requires excellent understanding and motivation from the individual, otherwise the decay will continue.

Once a lesion has cavitated, especially if dentin is involved, remineralization is much more difficult and a dental restoration is usually indicated ("operative treatment"). Before a restoration can be placed, all of the decay must be removed otherwise it will continue to progress underneath the filling.

Sometimes a small amount of decay can be left if it is entombed and there is a seal which isolates the bacteria from their substrate. This can be likened to placing a glass container over a candle, which burns itself out once the oxygen is used up. Techniques such as stepwise caries removal are designed to avoid exposure of the dental pulp and overall reduction of the amount of tooth substance which requires removal before the final filling is placed. Often enamel which overlies decayed dentin must also be removed as it is unsupported and susceptible to fracture (Ole, and Kidd, 2004).

Destroyed tooth structure does not fully regenerate, although remineralization of very small carious lesions may occur if dental hygiene is kept at optimal level. For the small lesions, topical fluoride is sometimes used to encourage remineralization. For larger lesions, the progression of dental caries can be stopped by treatment. The goal of treatment is to preserve tooth structures and prevent further destruction of the tooth. Aggressive treatment, by filling, of incipient carious lesions, places where there is superficial damage to the enamel, is controversial as they may heal themselves, while once a filling is performed it will eventually have to be redone and the site serves as a vulnerable site for further decay (King, 2011).

For children, preformed crowns are available to place over the tooth. These are usually made of metal (usually stainless steel but increasingly there are aesthetic materials). Traditionally teeth are shaved down to make room for the crown but, more recently, stainless steel crowns have been used to seal decay into the tooth and stop it progressing. This is known as the Hall Technique and works by depriving the bacteria in the decay of nutrients and making their environment less favorable for them. It is a minimally invasive method of managing decay in children and does not require local anesthetic injections in the mouth.

In certain cases, endodontic therapy may be necessary for the restoration of a tooth. Endodontic therapy, also known as a "root canal", is recommended if the pulp in a tooth dies from infection by decay-causing bacteria or from trauma. In root canal therapy, the pulp of the tooth, including the nerve and vascular tissues, is removed along with decayed portions of the tooth. The canals are instrumented with endodontic files to clean and shape them, and they are then usually filled with a rubber-like material called guttapercha. The tooth is filled and a crown can be placed. Upon completion of root canal

therapy, the tooth is non-vital, as it is devoid of any living tissue (Touger-Decker, and van Loveren, 2003).

An extraction can also serve as treatment for dental caries. The removal of the decayed tooth is performed if the tooth is too far destroyed from the decay process to effectively restore the tooth. Extractions are sometimes considered if the tooth lacks an opposing tooth or will probably cause further problems in the future, as may be the case for wisdom teeth. Extractions may also be preferred by people unable or unwilling to undergo the expense or difficulties in restoring the tooth (Touger-Decker, and van Loveren, 2003).

2.8 PREVENTION

To prevent dental caries there are four main principles: oral hygiene; healthy eating advice; increasing fluoride availability; and placement of fissure sealants (British Department of Health, 2007). Dental health education (i.e. information giving) is most beneficial on a one-to-one basis, and within the dental setting. Team work by all in the dental practice can potentially be very effective in this, particularly if a unified and consistent approach is used to deliver the preventive messages, which should be practical, personal and positive. Family support is also crucial, as to modify behaviours past the short-term the patient's parents or carers would need to create a supportive home environment for this modification to be sustained. (Scottish Intercollegiate Guidelines Network, 2005).

Oral Hygiene: Tooth brushing removes dental plaque, which is important to prevent gingivitis and periodontal disease, but for dental caries it is the fluoride in the toothpaste that has the important health benefit. Fluoride has a number of actions but most importantly is its topical action in inhibiting demineralisation and encouraging remineralisation of the tooth surface (Scottish Intercollegiate Guidelines Network, 2000).

Healthy eating advice: In the prevention of caries a key principle is that if a child or teenager has a diet where they frequently have foods and drinks that contain sugar, they increase their chances of getting decay. The aim should be to reduce the amount and also how often these foods and drinks are consumed. A useful initiating tool is a 3-day diet diary, which can be used to help motivate the patient. Advice is most helpful if it is constructive, so rather than 'banning' all confectionery the dental team should provide encouragement for healthier food choices, and give parents assistance in finding alternatives. A common misconception is that crisps are a 'healthier' snack; they tend to be high in salt and fats. Instead parents can encourage older

children to have sugar-free chewing gum, this not only encourages saliva production, which is protective, but if the gum contains xylitol it is also antibacterial (British Department of Health, 2007)

Increasing fluoride availability: The introduction of fluoride in toothpaste that occurred in the 1970s has been held responsible for the subsequent decrease in caries levels (Deery and Toumba, 2005). Oral health advice that concentrates on increasing fluoride use has been shown to be effective in reducing caries (Kay and Locker, 1998) There are now many products that contain fluoride available for either professional application or home use, such as fluoride rinses, supplements (tablets or drops) and varnishes. These are described in detail in the British Department of Health's Delivering better oral health document. There is evidence that children who use other forms of topical fluoride, such as a mouth rinse or varnish, as well as the use of fluoride toothpaste, will have an additional reduction in decay then if they used toothpaste alone (Marinho *et al.,*2004) Fluoride varnishes which are applied by a dental professional are an effective option. They have been shown to substantially reduce tooth decay in both the primary and permanent dentition (Marinho *et al.,* 2002). Unlike the use of a mouth rinse or supplement, the use of fluoride varnish does not depend on the patient's compliance at home. Application is also easy, with a small amount applied to dried tooth surfaces, and the patient's only requirement is then not to eat, drink or brush their teeth for 30 minutes. The regular use of a fluoride mouth rinse is also effective, and similarly fluoride tablets have a well-documented potential to inhibit caries, although both of these depend on the patient's use (Marinho *et al.,* 2002).

Placement of fissure sealants: The occlusal surfaces of permanent molar teeth are the sites most at risk of developing caries in children and teenagers, and actually represent almost 50% of the carious lesions in school children. A fissure sealant is a material that is placed in the pits and fissures to prevent caries development and they have been shown to work (Ahovuo-Saloranta *et al.,* 2004). Their use should be targeted at those patients who will benefit the most, such as those who are at an increased risk of developing caries (Ripa, 2013). The strongest predictor is a child's past caries experience, so if a patient had decay in their primary teeth they are then at greater risk of developing it in their permanent teeth and their first permanent molars should be fissure-sealed as soon as possible after their eruption. Their use does extend beyond permanent molars as they can also be placed on other susceptible

sites, such as palatal pits on upper anterior teeth and primary teeth.

3.0 MATERIALS AND METHOD

3.1 SAMPLE COLLECTION

Five cases was selected in the study between the age group of 18-25 years with visible depression on their tooth, discoloured or spoiled tooth and those who experience pains and have difficulty chewing/eating. Saliva samples were collected from the five persons in a sterile container using aseptic methods. The containers were labelled and the sample was transmitted to microbiology laboratory in science laboratory technology department in federal polytechnic Nekede, Owerri.

3.2 STERILIZATON OF MATERIALS

All the glass wares used for the practical was sterilized using the laboratory hot air oven at 160oc for 20 minutes. The glass spreader was spreader was sterilized by dipping it in 70% ethanol and passing it over Bunsen flame and the wire loop was sterilized by passing it through burning flame till it was red hot. The media used-nutrient agar, MacConkey agar, blood agar, Simmon citrate agar and triple sugar iron agar were prepared according to manufacturer's instruction and sterilized with the autoclave at 121oc for 15 minutes at 15 psi and allowed to cool to 45oc. The nutrient agar, MacConkey agar and blood agar were poured into sterile labelled petri dishes, 5 petri dishes for each agar. The petri dishes were left to cool and set for inoculation.

3.3 MICROBIAL ANALYSIS

Direct plating method was carried out using the spread plate technique. Normal saline was added to the different samples prior to plating. O.1ml of each sample was pipetted on three petri dishes, one nutrient agar plate, one MacConkey agar plate and one blood agar plate. A sterile bent glass rod was used to evenly spread the inoculum on each media. The inoculated plates were inverted and incubated at 37oc for 24hours.

3.4 MICROBIAL PLATE COUNT

After incubation, the different colonies formed on the plates were counted using the digital colony counter. The total population of the colonies was expressed as colony forming unit per milliliter (cfu/ml).

3.5 IDENTIFICATION OF BACTERIAL ISOLATES

The bacterial isolates from the plates were identified using their morphological appearance, Gram staining and other biochemical test.

3.6 GRAM STAINING TECHNIQUES

A smear of each of the bacterial isolates was made and fixed by air drying. The smears were covered with crystal violet stains and washed off after 60 seconds with water. The smears where then covered with lugol's iodine for 60seconds and washed off with water. The smears were decolourized with acetone alcohol and washed off after 3 seconds. The smears were then flooded with safranin and washed off after 2 minutes with clean water. The back of the slides were wiped and placed on a draining rack for air drying. Immersion oil was added to the smears and they were viewed under the microscope with x 100 oil immersion objective lens. Gram positive bacteria appeared pink.

3.7 BIOCHEMICAL TESTS

3.7.1 MOTILITY TEST

Wet mount preparation of each bacteria isolate was made, covered with a cover slip and viewed under the microscop for motility

3.7.2 CATALASE TEST

3ml of hydrogen peroxide was poured in a test tube and a colony of the test organism was collected with a sterite glass rod and immersed into the test tube. The production of bubbles indicate oxygen production which implies a positive test and the absences of bubbles production implies a negative test.

3.7.3 COAGULASE TEST

A drop of distilled water was placed on each end of a slide for each of the test organism. A colony of each test organism was emulsified in each of the drops of water to make two thick suspensions. A loopful of plasma was then added to one of the suspension and mixed gently for each test organism. Clumping within 10 seconds is an indication of positive test while no clumping is an indication of negative test.

3.7.4 OXIDASE TEST

A piece of filter paper was placed in a clean petri dish and three drops of freshly prepared oxidase reagent was dropped on the filter paper. A sterile piece of stick was used to remove a colony of the test organism and it was smeared on the oxidase reagent drop on the filter paper. The development of a blue- purple colouration is an indication of a positive test while none is an indication of a negative test.

3.7.5 CITRATE UTILIZATION TEST

A bacterial colony was inoculated in Simmon's citrate agar and incubated at 35-37^{0}c for 18- 24 hours. Thereafter, development of blue colour and visible growth on the slant is a positive test while

the absence of growth or colour change is a negative test.

3.7.6 SUGAR FERMENTATION TEST

Each colony of the test organisms were inoculated onto sterile agar slopes of triple sugar iron agar using stab inoculation. The inoculated agar slopes were incubated at 37^0c for 24hours. The different colours of the slopes and butts in addition to the presence of gas production and hydrogen sulphide (H_2S) blackening is indicative of the type of bacteria present.

3.7.7 SPORE STAINING

A smear of each of the bacterial isolates was made on a clean slide and fixed by air drying. The smears were then covered with malachite green and placed over steam for 5minutes while topping the slides with more malachite green when they are dried out. At the end of 5minutes, the smears were washed off with clean water and counter stained with safranin for 2minutes and washed off with water. The smears were allowed to dry before they were viewed with x100 oil immersion objective lens. Spore positive slides gave a co-appearance of pink and green colour while negative slides gave only pink colouration.

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

A total of 5 saliva samples was collected for this study and inoculated on different agar media. The morphological characteristics of the isolated microorganism prevalent on the media and the total viable count is shown in table 1

Table 1: Mean TVC and Morphological appearance

Media	Mean TVC	*Staphylococcus aureus*	*Streptococcus spp*
Nutrient agar	1.96×10^3	Small, circular, flat, non-mucoid golden raised yellow colonies	Very small, spherical raised center milkfish colonies.
Mac Conkey agar	1.65×10^2	Circular, slightly raised, pink colonies	Small, circular flat, non-mucoid colonies
Blood agar	1.63×10^3	Clear zone of haemolysis (β - haemolysis)	Small, circular convex colonies with different zone of haemolysis (α-haemolysis and γ-haemolysis)

Key TVC: Total Viable Count (cfu/ml)

The mean TVC was calculated by adding the different TVC for each media and dividing it by the total number of plates for the particular media. The morphological characteristics of the isolates was noticed to be same for different samples on the same media.

4.2 DISCUSSION

Dental caries and periodontal diseases may come into existence due to an alteration of the equilibrium of the oral bacterial population by many factors such as antimicrobial factors or the inhibitory substances of the human saliva (Goyette N, et al 1995). The pathogenic examination from the saliva sample in the present study have been shown to be important in the etiology of dental caries. Dental caries commonly known as oral microbial communities is one of the most complex bacteria flora associated with human body. So far more than 700 different bacterial species has been identified from human oral cavity and the majority of them are associated with dental caries (Christopher Dennis Packey, et al 2009).

In this study, a total of 5 samples were included from student within the age of 18-25 years with risk factors such as high sugar, consumption and irregular teeth brushing. Their teeth was also observed to have some symptoms of caries such as visible tooth depression with patches, pains and difficulty during chewing/eating. The samples showed growth on cultured media and gave characteristic identification features-heamolysis on blood agar.

The isolates identified included *Streptococcus spp* which showed gram positive cocci on stained smear arranged in chains, catalase negative and alpha haemolysis on blood agar. Also *Staphylococcus aureus* was isolated and clarified by gram staining – a gram positive cocci grouped in clusters, catalose test – positive, and beta haemolysis on blood agar.

From the different samples, *Staphylococcus aureus* was more isolated followed by *Streptococcus spp*. These organisms are part of the microbiota of the oral cavity (Cuesta Al. Jewtuchowicz, et al 2010). The presence of more isolated *S.aureus* and high concentration of *Streptococcus spp* is a more significant sign of chronic or acute dental infection (Cuesta Al. Jewtuchowicz 2010).

The prevalence of the isolated micro-organisms. The present result showing the prevalence of *Streptococcus spp* and *S.aureus* in the samples as a result of dietary patterns, i.e. consuming snacks and food containing high level of carbohydrate that fuel bacteria to produce acidic by-products which has a huge impact on the demineralization of enamel and development of caries, and irregular brushing pattern had shown the involvement of these organisms in dental caries.

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Dental caries is still a significant health concern with many bacteria involved in its formation and a lot of people suffering as a result. This study has concluded the involvement of *Staphylococcus aureus* and *Streptococcus spp* which are acidogenic and food containing high level of carbohydrate and irregular brushing pattern among the stimulation of growth of the oral microbes responsible for dental caries.

5.2 RECOMMENDATIONS

Healthy teeth and oral tissues and the need for oral health care are important for any section of society. Dental caries is an infectious microbial disease of multifactorial origin in which diet, host, and microbial flora interacts over a period of time in such a way so as to encourage demineralization of the tooth enamel with resultant caries formation. In view of this, the following recommendations are formulated to support this study.

- Proper and early enlightenment on dental caries for students across the nation from primary to tertiary levels.
- The access and efficient use of regular dental care both preventive and restorative should be ensured in dealing with this dental public health crisis.

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